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FORMULATION OF ANTIBACTERIAL MOUTHWASHES BASED ON AN AQUEOUS EXTRACT OF THE LEAVES OF *PROSOPIS AFRICANA* (GUILL. ET PERR.) TAUB (FABACEAE)

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Abstract

The aim of this work was to propose an antibacterial mouthwash formulation based on aqueous extracts of the leaves of *Prosopis africana* (Fabaceae) for maintaining oral hygiene. Three types of mouthwashes were formulated, incorporating the liquid extract, freeze-dried extract, and oven-dried liquid extract of *Prosopis africana* as the active ingredient. Quality control of the formulations involved the evaluation of macroscopic and organoleptic characteristics, pH, phytochemical composition, and in vitro antimicrobial activity. The mouthwashes were liquid in consistency, clear in appearance, homogeneously green in color, with a menthol odor and a sweet taste. The pH values ranged from 5.05 ± 0.03 for the mouthwash formulated with the liquid extract, 5.24 ± 0.1 for the mouthwash formulated with the oven-dried extract, and 5.18 ± 0.01 for the mouthwash formulated with the freeze-dried extract. The formulations containing the liquid extract showed higher levels of phytochemical content. All formulated mouthwashes exhibited broad-spectrum antimicrobial activity against the tested oral pathogens.

Keywords: Oral pathogens; *Prosopis africana* extracts; mouthwashes; Phytochemicals; Antimicrobial activity.

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Introduction

Oral diseases can be characterized as major public health problems because of their prevalence and high incidence in all regions of the world and because, as with all diseases, they mainly affect disadvantaged and socially marginalized populations [1]. According to the World Health Organization in 2022 [2], half of the world's population 45% or 3.5 billion people suffer from oral disease [3, 4]. These diseases usually result from dental biofilm, tartar and acid-forming bacteria [5]. Hygiene practices remain the best preventive measures against these conditions [6]. They excite several methods, tools and chemicals to maintain and preserve dental health, such as toothbrushes, mouthwashes and toothpastes [7].

Toothpastes have been around for decades and are one of the most important hygiene products when it comes to oral health [8]. It helps to remove dental biofilm more easily, prevents bad breath [9]. These products usually contain triclosan, chlorhexidine gluconate, fluoride, which can cause side effects such as enamel discoloration, taste alterations, etc. Which leads the dental products sector to face issues of naturalness. In fact, the trends and consumer desires that appeared for classic cosmetics a few years ago are also appearing for these oral products and this without neglecting the quality and effectiveness of these products. Moreover, conventional treatment of oral Diseases is extremely expensive in many industrialized countries and is inaccessible in most low- and middle-income countries.

This is how tooth pastes based on essential oils from plants which have proven their biological properties constitute therapeutic alternatives today in the

management of these oral diseases [10]. They act as antibiotics, analgesics, sedatives and anti-inflammatories in addition to being less likely to cause side effects [11].

Indeed, many previous studies have highlighted the beneficial properties of certain parts of medicinal plants in oral care [12]. Among these studies, plants such as clove, lavender, coconut (activated carbon), spearmint, propolis, *Zanthoxylum* (clavalier), *Prosopis africana*, etc. are used because of their anti-inflammatory, analgesic and antibacterial and antiseptic properties [5, 13]. Despite their traditional uses and demonstrated pharmacological properties, certain plants such as *Prosopis africana* have not yet been the subject of galenic formulations aimed at improving and/or optimizing their use in stable form. Thus, the aim of this work was to propose an antibacterial mouth wash formulation based on aqueous extracts of the leaves of *Prosopis africana* (Guill. Et Perr.) Taub (Fabaceae) to preserve oral hygiene. Thus, with the aim of offering a galenic form accessible to all, three types of extracts, namely freeze-dried, oven-dried and liquid obtained through different processes, were used in this work in order to retain the least expensive one.

Methodology

Plant Material

The leaves of *Prosopis African* as were harvested in the north-west of Burkina Faso. The plant material was identified by a botanist from the Institut de Recherche en Sciences de la Santé (IRSS) under the identification number BKMP 6L23. The leaves were dried in a ventilated room, protected from light and dust for two weeks at room temperature. The dried leaves were then crushed using a Gladiator type hammer mill and stored in airtight zip-lock bags.

Table 01: Excipients used in the preparation of mouthwashes

COMPONENTS	ROLE	SUPPLIERS
Aqua conservans	Solvent, antimicrobial preservative	IRSS, Burkina Faso
Distilled water	Solvent	IRSS, Burkina Faso
Propylene glycol	Humectant	CARLO ERBA, France
Prosopis africana extracts	Antibacterial	IRSS, Burkina Faso
Sodium lauryl sulfate	Foaming agent	CHEMICALSRORE
Sodium benzoate	Antimicrobial preservative	Sigma Aldrich
Methyl paraben	Antimicrobial preservative	Lot 20050219/A
Sucrose	Sweetener	Sigma Aldrich, USA
Mint essence	Flavoring	IRSS, Burkina Faso
Food coloring	Coloring	IRSS, Burkina Faso

Methods

Powder extraction

A decoction was made by dispersing 100 g of the powder in 1000 mL of distilled water contained in a flask brought to the boil for 30 minutes on a hotplate equipped with a refrigerating reflux system to prevent evaporation. After cooling, the mixture was first filtered and the filtrate was centrifuged at 2000 rpm for 5 min. The collected supernatant was filtered again using 150 mm diameter filter paper. The resulting decoction was divided into three parts. The first part constituted the liquid extract. The second part was dried in an oven at 65°C for 72 hours and the third part was frozen and then freeze-dried. The extraction yields were then determined

Pre-Formulation Study

Macroscopic and Organoleptic Characteristics

The macroscopic and organoleptic characteristics of extract (texture, color, taste, odor) were determined using the sense organs [14].

Residual Moisture Content (RMC)

The residual moisture levels of extract were determined using the method described by the European Pharmacopoeia 10th edition [14]. For each test, the measurement was carried out three times

pH

A pH meter determined the pH by immersing the electrode in 1% (m/v) aqueous solutions of each powder at 37°C. The mean value and the standard deviation were calculated (m±standard deviation, n = 3) (14).

Hygroscopicity

Hygroscopicity was determined using 1 g of extract according to the method described in European Pharmacopoeia 6.0(15). Extracts were introduced into suitable desiccators containing a saturated solution of ammonium chloride at 25 ° C. for 24 hours. The increase in mass allowed the calculation of the ratios expressed as a percentage.

Extract Solubility

The solubility was determined with the freeze-dried extract according to the method described by the European Pharmacopoeia 10th edition(14). For this, the extract was dissolved in solvents such as distilled water, absolute ethanol, 70% ethanol (V/V), absolute methanol, acetone, and chloroform, and appreciation was according to very soluble, soluble, unpleasant, slightly soluble, very slightly soluble and insoluble criteria.

Phytochemical Study

Dosage of some chemical molecules of interest

Total phenolics content

Total phenolic compounds were measured according to the method of Singleton *et al*. [16].

Total Flavonoids Content

The determination of flavonoids was carried out according to the method of Kumaran *et al*. [17].

Formulation

Formulation and Manufacturing Processes of Mouthwashes

Following the recommendations of European standards for mouthwash formulation, three formulations were produced. These formulations were also inspired by the work carried out by Taarabt *et al.* [18]. The qualitative and quantitative formulations of the bases of the formulated mouthwashes are presented in Table 02.

Tableau 02: Quantitative and qualitative formulation of mouthwash bases

Designation	Role	Formulation (gram)						
		F1	F2	F3	F4	F5	F6	F7
Lyophilized dry extract°	Active ingredient	0	0	0	0	1	0	0
Oven-dried extract°°	Active ingredient	0	0	0	0	0	1	0
Liquid extract°	Active ingredient	0	0	0	0	0	0	30
Propylene glycol	Humectant	30	20	20	20	20	20	20
Sodium lauryl sulfate	Foaming agent	0.5	0	0.5	0.5	0.5	0.5	0.5
Sodium benzoate	Preservative	1	1	0.2	0	0	0	0
Methylparaben	Preservative	0.5	0.2	0.2	0	0	0	0
Sucrose	Sweetener	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Food coloring	Coloring	0	0	0	0.5	0.5	0.5	0.5
Mint essence	Flavoring	2	2	2	2	2	2	2
Aqua conservans	Preservative	0	0	0	QSP 100	QSP 100	QSP 100	QSP 100
Distilled water	Solvent	Qsp 100	Qsp 100	Qsp 100	0	0	0	0

Quality Control of Mouthwashes

The quality control was carried out with the finished products, i.e. the most stable mouthwash absorption bases in which the freeze-dried, steamed and liquid extracts were incorporated.

Macroscopic and Organoleptic Characteristics

The aim was to determine the appearance, odor, color, taste, homogeneity and consistency.

pH

The pH of the mouthwashes was measured according to the extract method.

Dosage of some chemical molecules of interest in formulated

Total phenolics and flavonoids were assayed in mouthwashes according to the extract assay method.

Evaluation of in vitro antimicrobial activity of formulations

The *in vitro* antimicrobial activity of the mouthwashes was evaluated on the germs *Pseudomonas aeruginosa* ATTC 27653, wild *Pseudomonas aeruginosa*, *Candida albicans* ATTC 90028 and wild *Candida albicans*. Ciprofloxacin (15µg load) was used as a reference for *Pseudomonas aeruginosa* strains and Nystatin (100IU load) as a reference for *Candida albicans* strains. The swab seeding method was used. Muller-Hinton agar was used for bacteria while Savoured agar supplemented with chloramphenicol was used for the fungal strain. The results of the microbiological sensitivity tests were interpreted according to the criteria established by Ponce et *al.*, [19].These criteria make it possible to classify the sensitivity of target germs to raw plant extracts based on the diameters of the inhibition zones observed during the tests (D < 8 mm: resistant strain; 9 mm < D < 14: sensitive strain; 15 mm < D < 19 mm: very sensitive strain; D > 20 mm: extremely sensitive strain).

Statistical Analysis

Data were entered into Excel 2013. Results were expressed as the mean of three replicates with standard deviation (Mean±SD). They were analyzed by Graph Pad Prism 5 software. The series are considered significant when the probability of error (p) is lower than the accepted risk: 0.05 ($p<0.05$). The graphs were drawn with this same software.

Results

Physicochemical characteristics of the powder and the extract

Extraction Yields

The extraction yield was 17.79% for the freeze-dried extract and 16.63% for the oven-dried extract. The yield of the freeze-dried extract (17.79%) was significantly higher ($p<0.05$) than that obtained with the oven-dried extract (16.63%). This difference could be explained by the difference in drying methods [20]. These yields were lower than those of Bance *et al*. which were 19.2% with significant differences ($p<0.05$) [21]. Factors such as the difference in the locations where the leaves are harvested or the harvest period could explain these differences [22].

Macroscopic and Organoleptic Characteristics

Table 03 presents the macroscopic and organoleptic characteristics of the extracts.

Table 03: Macroscopic and organoleptic characteristics of the extracts

Characteristics	Freeze-Dried Extract	Oven Dried Extract	Liquid Extract
Color	Brownish	Brownish	Brownish
Smell	Characteristic	Characteristic	Characteristic
Taste	Bitter and astringent	Bitter and astringent	Bitter and astringent
Texture	Fine	Coarse	Liquid

The extracts had a brownish color and a strong characteristic or with a slightly bitter and astringent taste. The bitter taste could be due to the presence of alkaloids and flavonoids [21, 22]. The presence of tannins could explain the astringent taste of the extracts [23]. These results are in agreement with those reported by Haidara *et al*. who also found that the powder of the leaves of *Prosopis africana* was green and slightly bitter [24]. The very strong smell of the extract could be explained by the impact of certain environmental factors (temperature, humidity, etc.) [25]. These characteristics could allow the plant to be identified and characterized from other plants [26].

Residual moisture content (RMC)

The RMC of the extracts are presented in Table 04.

Table 04: RMC of the raw powder and dry extracts of the leaves of *Prosopis africana*

	Freeze-Dried Extract	Oven Dried Extract
RMC (%)	4.25±0.11	5.46±0.11

The RMC of the extracts were 4.25±0.11 and 5.46±0.11 for the lyophilize detract and the oven-dried extract, respectively. These values lower than 10%, constitute a favourable criterion for their conservation. Indeed, a high residual moisture content (≥10%) is favourable to certain enzymatic reactions that can lead to an alteration not only of the appearance of the substances, but also of their organoleptic properties and their therapeutic virtues [14, 23].

pH

The pH values of the different extracts are presented in Table 05.

Table 05: pH of the powder and the different extracts

	Freeze-dried extract	Oven dried extract	Liquid extract
pH	5.77±0.01	5.66±0.02	5.84±0.03

The lyophilized, oven-dried and liquid extracts had pH values of 5.77±0.01, 5.66±0.02 and 5.85±0.03, respectively. This slight difference could be due to a difference in the content of certain compounds between the extracts [24].

Hygroscopicity

The results of the hygroscopicity evaluation of the extracts are reported in Table 06.

Table 06: Hygroscopicity of *Prosopis africana* leaf extracts

	Freeze-dried extract	Oven dried extract
Hygroscopicity	17.23±0.025%	16.23±0.041%
Interpretation	Very hygroscopic	Very hygroscopic

The hygroscopicity value was 17.23±0.025% for the freeze-dried extract and 16.23±0.041% for the oven-dried extract. The hygroscopicity value was greater than 15% and indicates that both extracts are highly hygroscopic. Hence the need to store it in an airtight container and in a place with controlled humidity and handled in a low humidity environment [14].

Solubility of Extracts

The lyophilized and oven-dried extracts were highly soluble in 70% (v/v) hydroethanolic solution, soluble in distilled water, and poorly soluble in absolute ethanol and methanol. They were insoluble in acetone and chloroform. Since water is the solvent of choice for liquid pharmaceutical formulations, the solubility of the extracts in this solvent would allow a better release of the active ingredient [25]. Indeed, low aqueous solubility is the major problem encountered during the development of formulations [25, 26].

Phytochemical Study of Extracts

Contents of Secondary Metabolites of Interest

Table 07 presents the contents of total phenolics and flavonoids. The results are expressed in microgram Equivalent Tannic Acid (µgEAT)/mg for phenolic compounds and those of flavonoids are expressed in microgram equivalent quercetin (µgEQ)/mg.

Table 07: Content of secondary metabolites of interest in the extracts

Secondary Metabolites	Freeze-Dried Extract	Oven-Dried Extract	Liquid Extract
Total phenolics (µgEAT/mg)	72.34±1.24	59.72±4.91	77.64±0.07
Flavonoids (µgEQ/mg)	22.02±0.03	19.72±0.08	29.55±0.02

The total phenolic contents were 77.64 ± 0.07 µg EAT/mg, 72.34 ± 1.24 µg EAT/mg, and 59.72 ± 0.91 µg EAT/mg for the liquid, freeze-dried, and oven-dried extracts, respectively. As for flavonoids, the contents were 29.55 ± 0.02 µg EQ/mg for the liquid extract, 22.02 ± 0.03 µg EQ/mg for the freeze-dried extract, and 19.72 ± 0.08 µg EQ/mg for the oven-dried extract. The liquid extract had significantly higher phenolic and flavonoid contents than the freeze-dried and oven-dried extracts ($p < 0.05$). This may be attributed to the use of dry heat in the oven-drying method, which could have led to the degradation of some heat-sensitive secondary metabolites [27]. Previous studies conducted on aqueous extracts of the leaves of the same plant by Bance et al. and Mariko et al. reported lower total phenolic contents [21, 28]. These contents obtained in the extracts would serve as a reference for the quantification of these compounds in the formulations which will be produced [29].

Quality Control of Formulations

Macroscopic and Organoleptic Characteristics of Formulated Mouthwashes

The macroscopic and organoleptic characteristics of mouthwashes are shown in Table 08.

Table 08: Macroscopic and organoleptic characteristics of mouthwashes

Formulations	F1	F2	F3	F4	F5	F6	F7
Appearance	Viscous	Clear	Clear	Clear	Clear	Clear	Clear
Color	Brown	Brown	Brown	Green	Green	Green	Green

Odor	Menthol	Menthol	Menthol	Menthol	Menthol	Menthol	Menthol
Taste	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Consistency	Thick	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid

Mouthwash formulation F1 had a thick consistency due to the addition of xanthangum. Formulations F2 and F3 were clear liquids but F2 had a high amount of foam due to the amount of sodium lauryl sulfate. Formulation F3 had a brown color, a clear and homogeneous liquid appearance, with a minty odor and a sweet taste. Formulation F4 was green in color with a clear and homogeneous liquid appearance, a minty odor and a sweet taste. F4 was selected as the best formulation, for the incorporation of the different extracts, because of these characteristics and the use of a single antimicrobial preservative. The formulations each containing one of the 3 extracts (F5, F6 and F7) had the same characteristics.

Mouthwash pH

The pH values of mouthwashes are shown in Figure 2.

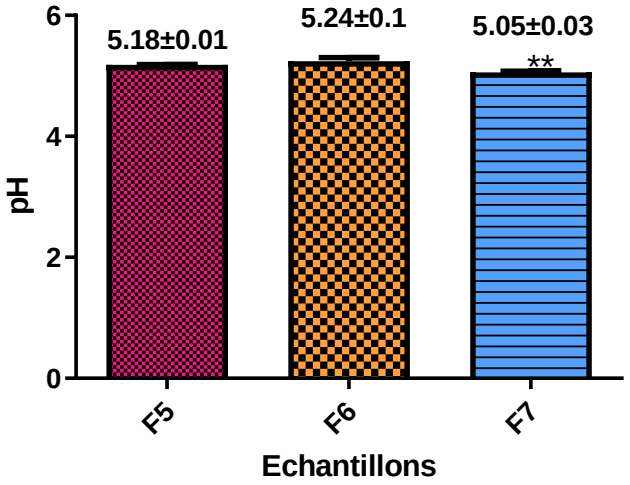

Figure 2: pH of mouthwashes

***: very significant difference compared to the reference ($p<0.05$)

The pH values ranged from 5.05±0.03 with F7 (mouthwash formulated with the liquid extract) to 5.24±0.1 with F6 (mouthwash formulated with the oven-dried extract). These pH values are lower than those of the pH of the extracts which varied from 5.66±0.02 with the steamed extract and 5.84±0.03 with the liquid extract. The pH of the formulations met the ideal pH standards for a mouthwash which would be between 4.0 and 6.5 [30].

Total Phenolic and Flavonoid Content of Mouthwashes

The total phenolic and flavonoid contents of the formulations are presented in Table 09.

Table 09: Total phenolic and flavonoid content of mouthwashes

Secondary Metabolites	F5	F6	F7
Total Phenolics (µgEAT/mg)	58.01±0.13**	47.24±3.27	59.91±0.5**
Flavonoids (µgEQ/mg)	18.73±0.59	17.57±0.4	21.40±0.29

F5: Mouthwash containing the lyophilized extract; F6: Mouthwash containing the oven-dried extract; F7: Mouthwash containing the liquid extract.

**: Significant difference compared to F6 ($p < 0.05$)

The total phenolic contents of the mouthwashes ranged from 47.24 ± 3.27 µg EAT/100 mg for the formulation with the oven-dried extract to 59.91 ± 0.5 µg EAT/100 mg for the formulation with the liquid extract. Flavonoid contents ranged from 17.57 ± 0.4 µg EQ/100 mg in formulations with the oven-dried extract to 21.40 ± 0.29 µg EQ/100 mg in those with the liquid extract.

Phytochemical analysis revealed higher concentrations in the formulations containing the liquid extract, while the lowest levels were observed in those containing the oven-dried extract. This suggests that the differences in formulation processes may not have significantly altered the levels of secondary metabolites. Therefore, these compounds could serve as reliable

markers for quality control of raw materials, intermediate products, and final formulations based on plant extracts [31]. Formulation with liquid extract could be preferred for formulation optimization.

In Vitro Antimicrobial Efficacy

The *in vitro* antimicrobial activity of mouthwashes containing the different types of extracts gave different inhibition diameters from one extract to another presented in Figure 3.

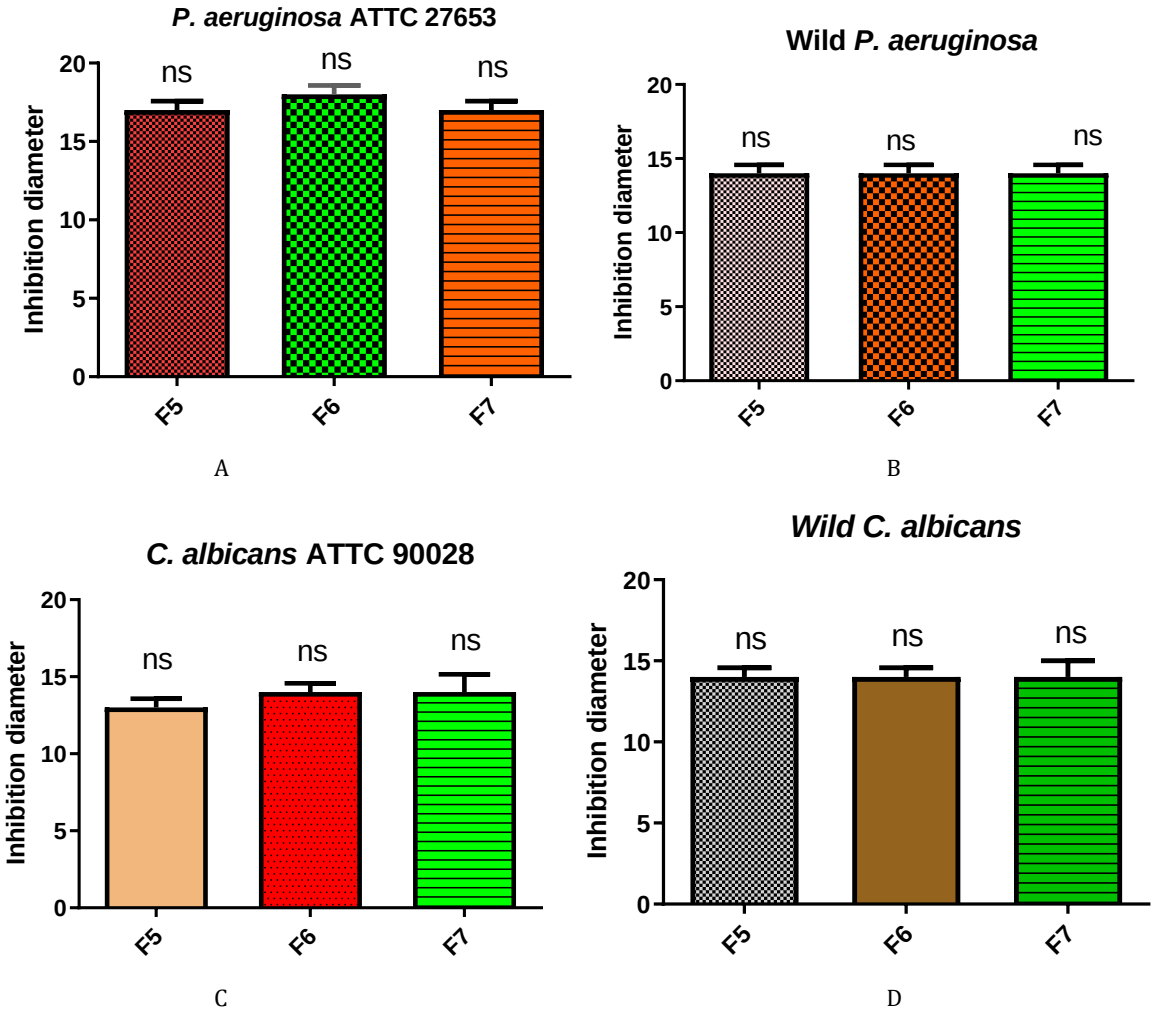

Figure 3: Inhibition diameters of mouthwashes on *P. aeruginosa* and *C. albicans*

A: Inhibition of mouthwashes on *P. aeruginosa* ATCC 27653
B: Inhibition of mouthwashes on wild *P. aeruginosa*
C: Inhibition of mouthwashes on *Candida albicans* ATTC 90028
D: Inhibition of mouthwashes on wild *Candida albicans*

The inhibition diameters of the mouthwash formulations were between 15 mm < D < 19 mm on the germ *Pseudomonas aeruginosa* ATTC 27653, between 9 mm < D < 14 for the germs *Pseudomonas aeruginosa* wild, *Candida albicans* ATTC 90028 and *Candida albicans* wild.

The inhibition diameters obtained for the different formulations made it possible to determine the sensitivity of the different germs to them as shown in Table 10.

Table 10: Evaluation of in vitro antimicrobial activity of formulations

Formulations / Strains tested	F5	F6	F7	Ref
***Pseudomonas aeruginosa* ATTC 27653**	VS	VS	VS	ES
Wild *Pseudomonas aeruginosa*	S	S	S	ES
***Candida albicans* ATTC 90028**	S	S	S	ES
Wild *Candida albicans*	S	S	S	ES

F5: mouth wash containing freeze-dried extract; F6: mouth wash containing steamed extract; F7: mouth wash containing liquid extract; Ref: reference antimicrobial substances. NS: non-sensitive; S: sensitive; VS: very sensitive; ES: extremely sensitive.

Pseudomonas aeruginosa ATCC 27653 was highly sensitive to all mouthwashes containing the three types of extracts. Similarly, other microorganisms such as *Candida albicans* ATCC 90028, wild strains of *Candida albicans*, and wild *Pseudomonas aeruginosa* were all sensitive to the different mouthwash formulations, with no statistically significant differences in zones of inhibition. The formulated mouthwashes demonstrated broad-spectrum antimicrobial activity, with zones of inhibition (ZOI) ranging from 13 to 18 mm against all tested oral pathogens. Both bacterial and fungal strains showed extreme sensitivity to Ciprofloxacin, the reference antibiotic. *Candida albicans* strains (ATCC 90028 and wild) were also sensitive to all three formulations and showed high sensitivity to Nystatin, the reference antifungal agent.

These results confirm that the extracts used in the mouthwash formulations exhibited antimicrobial activity against *Pseudomonas aeruginosa* and *Candida albicans*, as well as their biofilm [21, 32].

Conclusion

The aim of this study was to develop antibacterial mouthwash formulations based on aqueous extracts of *Prosopis africana* leaves obtained through three different extraction methods: freeze-drying, oven-drying, and direct use of the liquid extract. The three formulations were subjected to quality control assessments and evaluations of antimicrobial activity against microorganisms involved in oral diseases. Organoleptic and physicochemical analyses during quality control indicated that the formulations were stable. All three formulations exhibited similar characteristics, including liquid consistency, clear appearance, homogeneous green color, menthol odor, and sweet taste. The formulated mouthwashes demonstrated broad-spectrum antimicrobial activity against all tested oral pathogens. The results of this study are promising for the development of a new application of *Prosopis africana* extracts in oral healthcare. In particular, the use of liquid extracts shows potential for effective mouthwash formulation while reducing manufacturing costs by eliminating the freeze-drying step. This approach may serve as an alternative in support of the World Health Organization's recommendations for integrated oral health management tailored to the needs of individual countries.

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Ethical Clearance

Not applicable

Authors Contributions

All authors contributed similarly to manuscript writing, literature research, review design, literature analysis, and final text approval.

Conflict of Interest

The authors declared no conflict of interest for the given article

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